PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 0000054922	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/EP2004/011294	International filing date *(day/month/year)* 08 October 2004 (08.10.2004)	Priority date *(day/month/year)* 10 October 2003 (10.10.2003)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant BASF Plant Science GmbH		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 10 April 2006 (10.04.2006)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 740 14 35	Authorized officer Yolaine Cussac Telephone No. +41 22 338 70 80

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

26/4

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/011294	08.10.2004	10.10.2003

International Patent Classification (IPC) or both national classification and IPC
C12N9/02, C12N15/82, A01H5/00, C12P7/64

Applicant
BASF PLANT SCIENCE GMBH

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office - P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. +31 70 340 - 2040 Tx: 31 651 epo nl
Fax: +31 70 340 - 3016

Authorized Officer

Blanco Urgoiti, B

Telephone No. +31 70 340-4148

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2-12
	No:	Claims	1
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-12
Industrial applicability (IA)	Yes:	Claims	1-12
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The following documents are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

   D1: INUI H. ET AL.: 'PURIFICATION AND SOME PROPERTIES OF SHORT CHAIN-LENGTH SPECIFIC TRANS-2 ENOYL COENZYME A REDUCTASE IN MITOCHONDRIA OF EUGLENA-GRACILIS', JOURNAL OF BIOCHEMISTRY, 1986, vol. 100, no. 4, pages 995 to 1000

   D2: INUI H ET AL: 'FATTY-ACID SYNTHESIS IN MITOCHONDRIA OF EUGLENA-GRACILIS' EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 142, no. 1, 1984, pages 121-126.

   D3: OHLROGGE JOHN ET AL: 'Lipid biosynthesis' PLANT CELL, vol. 7, no. 7, 1995, pages 957-970.

2. NOVELTY(Art. 33(2) PCT)

2.1 D1 is considered to be the closest prior art. It discloses the purification of the trans-2-enoyl-CoA reductase from mitochondrial extracts of *Euglena gracilis*. It is described to consist of two different subunits (see fig. 1) and a total molecular weight of about 39 kDa. The activity of the purified protein is also checked.

2.2 The present application discloses the purification of the same enzyme as D1 checked to have identical activity, although found to have different molecular weights (see page 4, lns 25-34). In view of D1 the subject-matter of claim 1 is not novel under Article 33(2) PCT, because the amino acid sequence of the polypeptide of SEQ ID NO:2 is inherent to the nature of the protein of D1.

2.3 In view of the prior art the subject matter of claims 2-12 are novel in the sense of Article 33(2) PCT.

3. INVENTIVE STEP (Art. 33(3) PCT)

3.1 The problem to be solved by the subject matter of claim 2 can be formulated as the provision of the DNA sequence encoding the protein of D1. The solution is the provision of the nucleic acid of SEQ ID NOs:1,4, 6, 8 and 10. The provision of SEQ ID NO:1 is regarded as obvious because cloning the gene coding for a known protein is a routine laboratory practice for the person skilled in the art. Consequently the subject-matter of claim 2 cannot be considered as involving an inventive step in the sense of Article 33(3) PCT. The same is true for the expression cassettes of claims 7 to 9, and the genetically modified plants of claims 10 and 11.

3.2 The problem to be solved by the subject-matter of claim 3 is the provision of a method of increasing the oil content in a plant through the transgenic expression of the nucleic acid encoding a trans-2-enoyl-CoA reductase from *Euglena gracilis*. The solution given in the application consists of transforming Arabidopsis with the DNA of SEQ ID NO:4, which expresses the protein of SEQ ID NO:5. This protein lacks the first 126 amino acids being the "long putative mitochondrial targeting sequence of SEQ ID NO:2" and comprises instead, 28 amino acids "predicted to result in mitochondrial targeting" (see description page 54, lines 1-4). T2 transgenic plants show an increase of 10% in seed fatty acids compared to T2 plants transformed with an empty vector.

3.3 The protein desired to be expressed in the above mentioned method is part of a malonyl-CoA-independent fatty acid synthetic system (see D2), not described for higher plants (see D3 as a review of lipid biosynthesis in plants). It is therefore believed to be **not obvious** to obtain a higher oil content from transforming a plant with the gene of the application. Consequently, an inventive step could be acknowledged for the subject-matter of claim 3 (Art. 33(3) PCT), **only** in so far it relates to the protein of SEQ ID NO:5. The same would hold for dependent claims 4 to 6, and for claim 12.

3.4 The present application relates to DNA and amino acid sequences derived from a trans-2-enoyl-CoA reductase isolated from mitochondrial extracts of *Euglena gracilis* (SEQ ID NO:2). Example 6 tries to express it in an active form in *E. coli* without success. Therefore, a truncated version was generated (SEQ D NOs: 6/7), which was

actively expressed in *E coli*. Other variants, all comprising SEQ ID NO:6/7, have been generated: the above mentioned SEQ ID NOs:4/5; and SEQ ID NOs: 8/9 and 10/11, which have a plastid targeting sequence with or without the long putative mitochondrial targeting sequence of SEQ ID NO:2. All these different versions, although sharing amino acids 126 to 539 of SEQ ID NO:2, are targeted to either mitochondria, or plastids or not targeted to any organelle at all. In consequence, and for the same reasons stated in point 3.3, the problem to be solved by the subject-matter of claim 3 cannot be regarded as involving an inventive step (Art. 33(3) PCT) in so far the solution involves the sequences of SEQ ID Nos: 1, 6, 8 or 10, because a method involving the use of these sequences do not solve the problem. The same holds for dependent claims 4 to 6, and for claim 12.